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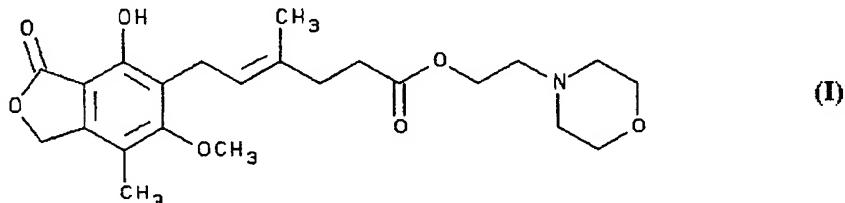
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : <b>C07D 307/88, A61K 31/535</b>		A1	(11) International Publication Number: <b>WO 95/07902</b> (43) International Publication Date: 23 March 1995 (23.03.95)
(21) International Application Number: PCT/US94/10142		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD).	
(22) International Filing Date: 12 September 1994 (12.09.94)			
(30) Priority Data: 08/121,841 15 September 1993 (15.09.93) US			
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(54) Title: CRYSTALLINE ANHYDROUS MYCOPHENOLATE MOFETIL AND INTRAVENOUS FORMULATION THEREOF

(57) Abstract

The crystalline anhydrous compound of formula (I) wherein the compound is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate, in particular the hydrochloride salt, and compositions, intravenous formulations, and a kit thereof.



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CRYSTALLINE ANHYDROUS MYCOPHENOLATE MOFETIL  
AND INTRAVENOUS FORMULATION THEREOF

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#### FIELD OF THE INVENTION

The present invention relates to 2-(4-morpholino)ethyl-E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate (hereinafter "MM"), particularly MM in its crystalline anhydrous salt form.

The invention also relates to intravenous formulations for the administration of MM, and a process for preparing the intravenous formulations using MM salt in its crystalline anhydrous form, where the salt is formed *in-situ* and lyophilized. The invention also relates to the use of such formulations in treating autoimmune disorders, psoriasis, inflammatory diseases (including in particular rheumatoid arthritis), tumors and viruses, and for immunosuppression, particularly for treatment of allograft rejection, especially including cardiac allograft rejection, pancreatic allograft rejection and renal allograft rejection, and for treating autoimmune diseases, including diabetes.

#### RELATED U.S. PATENTS

U.S. Patent No. 4,753,935, issued June 28, 1988, entitled "Morpholinoethyl Esters of Mycophenolic Acid and Pharmaceutical Compositions" discloses mycophenolate mofetil, the morpholinoethyl ester of mycophenolic acid, and certain simple ester derivatives thereof.

U.S. Patent No. 4,808,592, issued February 28, 1989, entitled "Method of Treating Diseases by Administering Morpholinoethyl Ester of Mycophenolic Acid and Derivatives Thereof" discloses a method of treating inflammatory and psoriatic diseases in mammals by administering a therapeutically effective amount of mycophenolate mofetil, the morpholinoethyl ester of mycophenolic acid, or certain simple ester derivative thereof.

U.S. Patent No. 4,952,579, issued August 28, 1990, entitled "Method of Treating Diseases by Administering Morpholinoethyl Ester of Mycophenolic Acid and Derivatives Thereof" discloses a method of treating malignant diseases in mammals by administering a therapeutically effective amount of mycophenolate mofetil, the morpholinoethyl ester of mycophenolic acid, or certain simple ester derivatives thereof.

U.S. Patent No. 4,786,637, issued November 22, 1988, entitled "Treatment of Allograft Rejection with Mycophenolic Acid Morpholinoethyl Ester and Derivatives Thereof" discloses a method of treatment of allograft rejection in mammals by administering a therapeutically effective amount of mycophenolate mofetil, the morpholinoethyl ester of mycophenolic acid, or certain simple ester derivatives thereof.

U.S. Patent No. 4,948,793, issued August 14, 1990, entitled "Treatment of Autoimmune Diseases With The Morpholinoethyl Ester of Mycophenolic Acid, and Derivatives Thereof" discloses a method of treating autoimmune diseases by administering a therapeutically effective amount of mycophenolate mofetil, the morpholinoethyl ester of mycophenolic acid, or certain simple ester derivatives thereof.

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U.S. Patent No. 4,992,467, issued February 12, 1991, entitled "Treatment of Autoimmune Diseases With Mycophenolic Acid, and Derivatives and Formulations Thereof", which discloses a method of treating autoimmune diseases in mammals by administering a therapeutically effective amount of certain simple phenolic esters of mycophenolic acid.

5 All of the aforementioned U.S. patents are hereby incorporated by reference.

#### BACKGROUND INFORMATION

10 Mycophenolic acid is a weakly-active antibiotic found in the fermentation broth of *Penicillium brevi-compactum*. Derivatives of mycophenolic acid, in particular, the morpholinoethyl ester thereof [i.e., 2-(4-morpholino)ethyl-(E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride] and certain simple ester derivatives of the phenolic hydroxyl group have been found to be effective in the treatment autoimmune disorders, psoriasis, inflammatory 15 diseases (including in particular rheumatoid arthritis), tumors, viruses, allograft rejection, especially including cardiac allograft rejection, pancreatic allograft rejection and renal allograft rejection, and autoimmune diseases, including diabetes. When compared with mycophenolic acid, these ester derivatives show advantageous pharmacokinetic properties which enhance the systemic introduction of mycophenolic acid, for example, solubility in the delivery environment (e.g., the stomach), peak plasma concentration, maximum plasma concentration, and improved activity, e.g., anti-inflammatory activity.

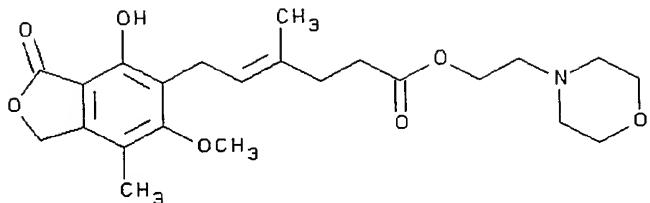
20 Administration of therapeutic agents by intravenous formulation is well known in the pharmaceutical industry. An intravenous formulation should possess certain qualities aside from being just a composition in which the therapeutic agent is soluble. For example, the formulation should promote the overall stability of the active ingredient(s), also, the manufacture of the formulation should be cost effective. All of these 25 factors ultimately determine the overall success and usefulness of an intravenous formulation.

30 MM in its monohydrate salt form is more stable than MM in its amorphous salt form, however the amorphous salt form has better solubility 35 characteristics. It has been surprisingly found that a newly discovered crystalline anhydrous salt form of MM possesses about a two-fold increase in solubility over the monohydrate salt form, while possessing the same stability characteristics of the monohydrate salt form.

#### SUMMARY OF THE INVENTION

40 One aspect of the invention relates to the crystalline anhydrous compound of the formula,

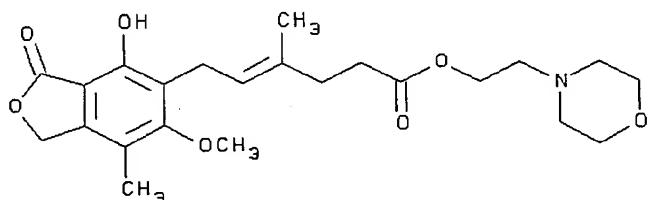
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(i.e., MM) wherein MM is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate.

10 Another aspect of the invention relates to the crystalline anhydrous form of MM HCl, i.e., 2-(4-morpholino)ethyl-(E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

15 Another aspect of the invention relates to a composition suitable for preparing an aqueous intravenous formulation which comprises MM, i.e., Formula I,



Formula I

25 wherein MM is complexed as a pharmaceutically acceptable salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate and the pharmaceutically acceptable salt is in its crystalline anhydrous form and pharmaceutically acceptable excipients.

30 Yet another aspect of the invention relates to a process of manufacturing the composition, where the solution from which the composition is derived is adjusted to a pH in the range of 3.2 to 3.6, the salt is formed *in situ*, and the solution lyophilized, resulting in the compound of Formula I in its crystalline anhydrous form.

35 Another aspect of the invention is the use of a compound of Formula I above for the treatment of disease, such as autoimmune disorders, psoriasis, inflammatory diseases (including in particular rheumatoid arthritis), tumors, viral diseases, autoimmune diseases including diabetes, allograft rejection, especially including cardiac allograft rejection, pancreatic allograft rejection and renal allograft rejection.

40 Another aspect of this invention is a kit useful for preparing an intravenous formulation comprising a) a composition suitable for preparing an aqueous intravenous formulation which comprises a compound of Formula I above wherein the compound is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate, and

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said salt is in a crystalline anhydrous form; and pharmaceutically acceptable excipients; and b) an appropriate amount of a liquid medium.

#### BRIEF DESCRIPTION OF THE FIGURES

5 Figure IA shows the X-ray diffraction patterns of the crystalline monohydrate form of MM HCl.

Figure IB shows the X-ray diffraction patterns of the crystalline anhydrous form of MM HCl.

10 Figure IIA shows the X-ray diffraction patterns of the composition containing the amorphous form of MM HCl.

Figure IIB shows the X-ray diffraction patterns of the composition containing the crystalline anhydrous form of MM HCl with polysorbate 80.

Figure IIC shows the X-ray diffraction patterns of the composition containing the crystalline anhydrous form of MM HCl without polysorbate 80.

15 Figure IIIA shows the Differential Scanning Calorimetry (hereinafter "DSC") thermogram for the amorphous form of MM HCl.

Figure IIIB shows the DSC thermogram for the crystalline anhydrous form of MM HCl after the lyophilization process.

#### DETAILED DESCRIPTION OF THE INVENTION

##### Definitions

20 The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

25 As used herein, the term "pharmaceutically acceptable salt" refers to a compound that is ionically complexed to an anion, such as, chloride, sulfate, citrate, acetate, phosphate, maleate and/or mesylate. Within the present disclosure, the compounds of Formula I can be complexed with various anions to form the corresponding pharmaceutically acceptable salt, e.g., hydrochloric acid and the morpholinoethyl ester of mycophenolic acid can form the pharmaceutically acceptable salt, 2-(4-morpholino)ethyl-(E)-30 6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

35 As used herein, the term "crystalline form" or "crystal form" means that a certain material has definite shape and an orderly arrangement of structural units, which are arranged in fixed geometric patterns or lattices.

As used herein, the term "crystalline monohydrate" or "monohydrate crystalline" means that the crystalline form of a certain material includes a single molecule of water.

40 As used herein, the term "crystalline anhydrous" or "anhydrous crystalline" means that the crystal form of a certain material contains no molecule of water.

As used herein, the term "amorphous form" means that a certain material has no definite shape and no orderly arrangement of structural units.

45 As used herein, the term "pharmaceutically acceptable excipients"

refers to those materials that are acceptable for use in pharmaceutical formulations, and are added to the formulation to promote the stability and viability the formulation (e.g., bulking agents, clarifying agents and buffering agents).

5 As used herein, the term "bulking agent" refers to compounds, such as, dextrose, mannitol and/or sucrose that are used to act as bulk, provide the matrix structure and stabilize a formulation, i.e., to slow or prevent decomposition of the therapeutic agent.

10 As used herein, the term "clarifying agent" refers to surfactant type compounds, such as polysorbate 80, which are used to reduce the haziness of a solution.

15 As used herein, the term "buffering agent" refers to compounds which resist change in pH when  $H^+$  or  $OH^-$  is added. A buffering agent is most resistive to change at the pH of the exact midpoint of its titration curve, i.e., where the concentration of the proton acceptor equals that of the proton donor and the pH is equal to the  $pK'$ . A buffering agent can be a single compound, e.g., citric acid, or a combination of compounds.

20 As used herein, the term "q.s." means adding a quantity sufficient to achieve a certain state (e.g., volume, i.e., to bring a solution to a desired volume).

25 As used herein, the term "q.s. to pH" means the addition of acid or base in a quantity sufficient to bring a solution to a desired pH (e.g., q.s. to pH 3.4 means the addition of acid or base to bring the solution to a pH of 3.4).

As used herein, the term "WFI" refers to Water for Injection that meets specifications set forth in the U.S. Pharmacopeia (U.S.P.).

30 As used herein, the term "ramp rate" refers to the rate at which the temperature is changed (i.e., increased or decreased) over a temperature range.

35 The term "reconstitute" refers to the process where a composition is combined with an appropriate liquid medium, e.g., water (WFI), 5% dextrose solution, or saline to yield an intravenous solution.

As used herein, the term "treatment" or "treating" means any treatment of a disease in a mammal, and includes:

35 (i) preventing the disease, i.e., causing the clinical symptoms of the disease not to develop;

• (ii) inhibiting the disease, i.e., arresting the development of clinical symptoms; and/or

40 (iii) relieving the disease, i.e., causing the regression of clinical symptoms.

45 As used herein, the term "therapeutically effective amount" refers to that amount of MM, which when administered to a mammal in need thereof, is sufficient to effect the stated treatment. The amount that constitutes a "therapeutically effective amount" will vary depending on the compound, the condition or disease and its severity, and the mammal to be treated, but

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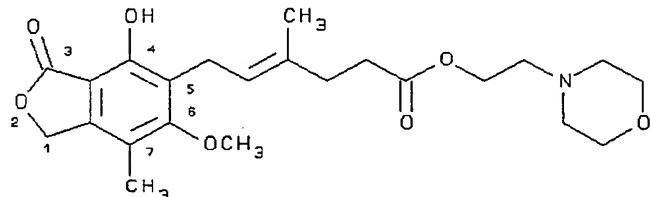
may be determined routinely by one of ordinary skill in the art with regard to contemporary knowledge and to this disclosure.

As used herein, the term "%w/v" means the percentage weight (gm) of a single ingredient relative to the total volume of the entire formulation, for example, 500 mg of an ingredient in a total volume of 8 ml is 6.25 %w/v, or 500 mg of an ingredient in a total volume of 5 ml is 10 %w/v.

As used herein, the term "%v/v" means the percentage volume of a single ingredient relative to the total volume of the entire formulation, for example, 1.17 ml of an ingredient in a total volume of 8 ml is 14.6 %v/v, or 1.17 ml of an ingredient in a total volume of 5 ml is 23.4 %v/v.

#### Nomenclature

The structure and numbering convention for mycophenolate mofetil "MM", also known as the morpholinoethylester of mycophenolic acid [i.e., 2-(4-morpholino)ethyl-E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate], is as follows:



Formula I

#### STARTING MATERIALS

The morpholinoethyl ester of mycophenolic acid and certain simple ester derivatives thereof are prepared according to U.S. Patent No. 4,753,935, issued June 28, 1988, entitled "Morpholinoethyl Esters of Mycophenolic Acid and Pharmaceutical Compositions", previously incorporated by reference, or described in co-pending Serial No. 07/993,146, filed, December 18, 1992, now U.S. Patent No. 5,247,083, incorporated herein by reference.

Polysorbate 80 is sold under the tradename of "Tween 80" and is obtained from ICI Americas, Inc., Wilmington, Delaware.

Citric acid, sodium hydroxide (NaOH) and hydrochloride acid (HCl) of suitable purity are obtained from the Aldrich Chemical Company.

Water for injection (WFI) indicates water meeting the purity standards set forth in the U.S. Pharmacopeia for injectable solutions.

#### OVERVIEW OF THE PREPARATION OF THE FORMULATIONS

The process by which the ingredients of a formulation are combined is known as "compounding", which yields the bulking solution. The bulking solution is lyophilized [i.e., the process where water and/or solvent (e.g., methanol, ethanol, or isopropanol) are removed with decreased pressure and varied temperature conditions] yielding a "composition". The intravenous formulation is reconstituted at the time of use from the

composition with a suitable liquid medium. The intravenous formulation is then administered.

**PREPARATION OF A COMPOSITION WHERE MM HCl IS IN ITS CRYSTALLINE ANHYDROUS FORM**

5                   **Compounding Process**

About 23 to 25.3 %w/v of an acid, preferably about 24.4 %w/v of 1N HCl (if concentrated HCl is used, a proportionally smaller % is used), and about 0.05 to 0.5 %w/v, preferably about 0.1 %w/v, of citric acid is added to a volume of water suitable for injection (WFI) approximating 50% of the 10 final volume of the bulking solution [e.g., for a final bulking solution volume of 10 liters, 5.0 liters of water (WFI) is used]. To this solution is dissolved about 0.01 to 2 % w/v, preferably about 0.5 %w/v, of a 15 clarifying agent, such as, polysorbate 80, and about 10 %w/v of MM; and about 0.10 to 10 %v/v, preferably about 3.2 %v/v, of ethanol. The pH of the 20 solution is adjusted to a pH of about 3.4 using 1N HCl or 2N NaOH, as appropriate. A sufficient amount of water suitable for injection (WFI) is added to bring the volume of the bulking solution to the desired final volume (e.g., for a 2000 - 20 cc vial batch with 5 cc of solution per vial, the final volume of the bulking solution is 10 liters). The pH of the solution is again adjusted to a pH of about 3.4. Under aseptic conditions, the solution is filtered (e.g., using a 0.2  $\mu$ m membrane filter).

The bulking solution is loaded into individual vials, where the volume of solution loaded is about 25% of the volume of the vial (e.g., for a 20 cc vial, about 5 ml of bulking solution is loaded), and the vials are 25 partially stoppered with a lyo-stopper (i.e., a stopper suitable for lyophilization) to allow for lyophilization of the solution.

**Lyophilization Cycle For Crystalline Anhydrous Form**

The lyo-stoppered vials containing the bulking solution are 30 lyophilized according to the following procedure (the temperature is measured from the shelf where the vials are placed within the lyophilization chamber):

a.       The temperature is equilibrated to a range of about 22°C to about 28°C, preferably about 25°C, prior to the introduction of the vials. The temperature is maintained during the loading of the vials. After loading 35 is completed, the temperature is maintained for a period of about 10 to 30 minutes, preferably about 20 minutes.

b.       The temperature is decreased to a range of about 5°C to -15°C, preferably about -10°C, in a period of about 1 to 3 hours, preferably about 2 hour 20 minutes (i.e., at a ramp rate of about -0.25°C/min).

c.       The temperature is maintained in a range of about 5°C to -15°C, preferably about -10°C, for a period of about 1 hour 30 minutes to 3 hours and 30 minutes, preferably for a period of about 2 hours 30 minutes.

d.       The temperature is decreased to a range of about -37°C to -43°C, preferably about -40°C, over a period of 1 to 3½ hours, preferably 45 about 2 hours, (i.e., at a ramp rate of about -0.25°C/min).

e. The temperature is maintained in a range of about -37°C to -43°C, preferably about -40°C, for about 3 to 7 hours, preferably about 5 hours.

5 f. The lyophilization chamber pressure is reduced to a range of about 70 to 130 mTorr, preferably about 100 mTorr.

g. After the desired lyophilization chamber pressure is reached, the temperature is maintained at a range of about -20 to -60°C, preferably about -40°C, for a period of about 3 to 7 hours, preferably about 5 hours.

10 h. The temperature is increased to a range of about -13° to -19°C, preferably about -16°C, over a period of about 6 to 10 hours, preferably about 8 hours (i.e., at a ramp rate of about 0.05°C/min) and maintained at this temperature for an additional period of about 8 to 12 hours, preferably about 10 hours.

15 i. The temperature is increased to a range of about 50°C to 78°C, preferably about 70°C, over a period of about 5 to 9 hours, preferably about 7 hours and 10 minutes (i.e., at a ramp rate of about 0.20°C/min) and maintained at this temperature until Step k. is completed.

20 j. The temperature is maintained until all the product thermocouples (i.e., temperature sensors attached to the vials) are at a temperature in the range of about 57°C to 63°C for a period of at least 7 to 13 hours.

25 k. The temperature is decreased to a range of about 22°C to 28°C, preferably about 25°C, over a period of about 2 to 4 hours, preferably about 3 hours (i.e., at a ramp rate of about -0.25°C/min) and maintained at this temperature for a period of about 1 to 3 hours, preferably about 2 hours.

30 l. The lyophilization chamber pressure is slowly increased to a pressure in a range of about 3 to 12 psi, preferably about 4 to 8 psi, more preferably about 4 psi, using nitrogen (NF) over a period of no less than about 15 minutes. The vials are stoppered under partial pressure.

35 m. The lyophilization chamber pressure is increased to atmospheric pressure. If it is not practical to unload the chamber after seating the stoppers, the vials may be kept at a temperature in the range of 23 to 27°C, preferably about 25°C, for a maximum of up to 24 hours. The vials are crimped sealed.

The composition is stored in the sealed vials until reconstitution at time of use.

#### PREPARATION OF THE INTRAVENOUS FORMULATION FOR ADMINISTRATION

40 The intravenous formulation is prepared by reconstituting the composition described previously with an appropriate liquid medium, such as, water for injection (WFI) or 5% dextrose solution. A desired concentration of the intravenous formulation can be obtained by reconstituting an appropriate amount of the composition with an appropriate volume of liquid medium. A desired concentration of the intravenous formulation provides a therapeutically effective amount of MM to the animal

in need of the intravenous pharmaceutical formulation of this invention and maintains a therapeutically effective level of the active ingredient in the animal. The dose which is therapeutically effective will depend on the rate at which the intravenous formulation is delivered to the animal and the concentration of the intravenous formulation. For example, two vials containing a composition [e.g., 500 mg of MM per vial (which is equivalent to 542 mg of MM HCl)] are reconstituted with a 5% dextrose solution (14 ml of 5% dextrose solution per vial) yielding a total of 28 ml of solution. The reconstituted solution is incorporated into dextrose solution in an infusion bag and q.s. to 166 mL, resulting in a solution containing 6 mg/ml of MM, suitable for intravenous infusion administration. The preferred concentration of MM in the liquid medium, in the infusion bag, is about 3 to about 10 mg/ml, preferably about 5 to about 6 mg/ml.

Solutions show no significant loss of label strength (%LS) over 24 hours after being prepared and stored at 25°C. It should be noted that good pharmacy practice dictates that the reconstituted solution should be administered immediately upon reconstitution, however, the stability of the reconstituted solution is not adversely affected if administration is delayed.

The composition of this invention can be provided to the dispensing person, e.g., a pharmacy or hospital, in the form of a vial containing the composition, or in the form of a kit comprising a vial or vials containing the composition and an appropriate amount of a liquid medium.

#### **PREFERRED EMBODIMENTS**

Most preferred is the crystalline anhydrous form of the hydrochloride salt of the morpholinoethyl ester of mycophenolic acid, i.e., 2-(4-morpholino)ethyl-E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

Also most preferred is the composition which comprises MM HCl in a crystalline anhydrous form and pharmaceutically acceptable excipients.

#### **PREFERRED PROCESSES**

A preferred process of compounding (i.e., preparation of the bulking solution) is where the temperature is maintained at about 21°C to 33°C.

An especially preferred process of compounding is where the temperature is maintained at about 25°C ± 2°C.

A preferred process of compounding is where the pH of the bulking solution is adjusted to 3.4 ± 0.5.

An especially preferred process of compounding is where the pH of the bulking solution is adjusted to 3.4 ± 0.2.

Preferred is a lyophilization cycle for the preparation of a pharmaceutical composition with the crystalline anhydrous form of MM HCl where the temperature is decreased from 25°C to a temperature in the range of 5 to -15°C, where a ramp rate of -0.25°C/min ± 0.1°C.

Most preferred for the temperature range of 25°C to -10°C is where the ramp rate is -0.25°C/min ± 0.05°C.

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Preferred is a lyophilization cycle for the preparation of a pharmaceutical composition with the crystalline anhydrous form of a MM HCl where the temperature is decreased from a temperature in the range of 5°C to a temperature in the range of -15°C to -40°C, where the ramp rate is 5 -0.25°C/min ± 0.1°C.

Most preferred for the temperature range of -10°C to -40°C where the ramp rate is -0.25°C/min ± 0.05°C.

Preferred is a lyophilization cycle for the preparation of a pharmaceutical composition with the crystalline anhydrous form of MM HCl where the temperature is increased over a range of -16°C to 70°C where the ramp rate is 0.20°C/min ± 0.1°C.

Most preferred for the temperature range of -16°C to 70°C where the ramp rate is 0.20°C/min ± 0.05°C.

#### UTILITY

The present invention is useful for the treatment of disease, such as autoimmune disorders, psoriasis, inflammatory diseases (including in particular rheumatoid arthritis), tumors, viral diseases, autoimmune diseases including diabetes, allograft rejection, especially including cardiac allograft rejection, pancreatic allograft rejection and renal allograft rejection.

#### EXAMPLES

The following examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

#### EXAMPLE 1

##### PREPARATION OF THE MYCOPHENOLATE MOFETIL HYDROCHLORIDE IN ITS CRYSTALLINE ANHYDROUS FORM

###### 1A. Preparation of Crystalline Anhydrous MM Complexed With Chloride

Mycophenolate mofetil hydrochloride is prepared as described in U.S. Patent No. 4,753,935. E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate (38.0 g) was dissolved in isopropanol (200 ml) and the solution was added to a solution of hydrogen chloride (10.0 g) in isopropanol (150 ml). The hydrochloride salt was collected by filtration and dried under vacuum (m.p. 154-155°C).

The crystalline anhydrous form of mycophenolate mofetil hydrochloride is prepared by heating the crystalline monohydrate hydrochloride form of MM HCl at 60°C for 30 minutes. The crystalline anhydrous form of the compound was confirmed by X-ray crystallography as shown in Figure 1B.

The crystalline anhydrous form of mycophenolate mofetil hydrochloride was found to have a solubility (when viewed over 3 days) of approximately 84 mg/ml in typical intravenous formulation solutions (e.g., 5% dextrose solution or water suitable for injection) versus approximately 40 mg/ml for the crystalline monohydrate form.

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1B. Preparation of Crystalline Anhydrous Salt of MM Varying the Anion  
 By following the procedures described in Example 1A and varying the anion of the starting material with the following compounds,  
 crystalline monohydrate form of mycophenolate mofetil sulfate,  
 crystalline monohydrate form of mycophenolate mofetil phosphate, and  
 crystalline monohydrate form of mycophenolate mofetil acetate,  
 there are obtained the following compounds,  
 crystalline anhydrous form of mycophenolate mofetil sulfate,  
 crystalline anhydrous form of mycophenolate mofetil phosphate, and  
 crystalline anhydrous form mycophenolate mofetil acetate.

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## EXAMPLE 2

PREPARATION OF MANUFACTURING BATCH OF  
 COMPOSITION OF MYCOPHENOLATE MOFETIL HCl  
 IN ITS CRYSTALLINE ANHYDROUS FORM

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2A. Preparation of Manufacturing Batch of 2000 - 20 cc vials using 1N HCl  
 Ingredient quantity/vial

Mycophenolate Mofetil	500.0 mg
1 N HCl	1220.0 mg
Citric Acid Anhydrous	5.0 mg
Polysorbate 80	25.0 mg
Ethanol (95%)*	0.16 ml
WFI* qs	5.0 ml
1N HCl/NaOH qs to pH 3.4	

\*Removed during lyophilization

30 A batch (manufacturing scale, 2000 - 20 cc vials) of the composition containing 542 mg of MM HCl (i.e., 500 mg of MM) in its crystalline anhydrous form per vial was prepared.

The ingredients were combined according to the following procedure.

1. 2440 gm of 1 N HCl and 10 gm of citric acid were combined with a sufficient amount of water suitable for injection (WFI) to obtain a volume of 7.5 liters.
2. 50 gm of Tween 80 was dissolved in the solution from step 1.
3. 1000 gm of MM was dissolved in the solution from Step 2.
4. 320 ml of ethyl alcohol was added to the solution of Step 3.
5. The pH of the solution from Step 4 was adjusted to pH 3.4 ( $\pm$  0.2).
- 40 6. Sufficient water (WFI) was added to bring the volume of the solution to 10 liters.
7. Under aseptic conditions, the solution was filtered using two 10" Millipore Durapore 0.2  $\mu$ m filter (CVGL).
8. 5 ml of the filtrate from Step 7 was loaded into 2000 - 20cc multi-dose vials (i.e., 5 ml of filtrate per 20cc vial) and partially stoppered with lyo stoppers.

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9. The vials were loaded into a lyophilizer where the temperature was stabilized to 25°C (Dura-Stop MP Freeze Dryer, FTS system).
10. Lyophilization Cycle
  - 5 a. The temperature was measured from the shelf where the loaded vials were placed, and thermocouples were fitted to various vials to measure their temperature.
  - b. The lyophilization chamber temperature was equilibrated at 25°C. The loaded vials were introduced into the lyophilization chamber and the temperature was maintained at 25°C for 20 minutes.
  - 10 c. The shelf temperature was decreased from 25°C to -10°C over a period of 2 hours 20 minutes (Ramp rate: -0.25°C/min).
  - d. The temperature was maintained at -10°C for 2.5 hours.
  - e. The temperature was decreased to -40°C over 2 hours (Ramp rate: -0.25°C/min).
  - 15 f. The temperature was maintained at -40°C for 5.0 hours.
  - g. The lyophilization chamber pressure was reduced to 100 mTorr.
  - h. After the pressure reached 100 mTorr (approximately 30 minutes), the temperature was maintained at -40°C for an additional 5 hours.
  - 20 i. The temperature was increased to -16°C over 8 hours (Ramp rate: 0.05°C/min.) and maintained at -16°C for an additional 10 hours.
  - j. The temperature was increased to 70°C over 7 hours 10 minutes (Ramp rate: 0.20°C/min) and maintained at 65°C until all the thermocouples indicated a temperature of 60°C for at least 7 hours (total time of approximately 11 hours).
  - 25 k. The temperature was decreased to 25°C over 3 hours (Ramp rate: -0.25°C/min) and maintained at 25°C for 2 hours.
  - l. The lyophilization chamber pressure was gradually increased to 7.5 psi using Nitrogen (NF) over a period of no less than 15 minutes.
  - 30 m. The vials were stoppered under partial pressure.
  - n. The lyophilization chamber pressure was increased to atmospheric pressure using filtered air.
  11. The vials were then sealed.
- 35 Characteristic analytical data confirms that the resulting composition contains MM HCl in a crystalline anhydrous form (having X-ray crystallography data as shown in Fig IIB).

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## 2B. Preparation of Manufacturing Batch of 2000 - 20 cc vials using concentrated HCl

Ingredient	quantity/vial
5 Mycophenolate Mofetil	500.0 mg
concentrated HCl	119.25 mg
Citric Acid Anhydrous	5.0 mg
Polysorbate 80	25.0 mg
Ethanol (95%)*	0.16 ml
10 WFI* qs	5.0 ml
1N HCl/NaOH qs to pH 3.4	

\*Removed during lyophilization

15 A batch (manufacturing scale, 2000 - 20 cc vials) of the composition containing 542 mg of MM HCl (i.e., 500 mg of MM) in its crystalline anhydrous form per vial was prepared.

The ingredients were combined according to the following procedure.

1. 119.25 gm of concentrated HCl were combined with a sufficient amount of water suitable for injection (WFI) to obtain a volume of 7.2 liters. 10 gm of citric acid were dissolved in the HCl solution.
2. 50 gm of Tween 80 was dissolved in the solution from step 1.
3. 1000 gm of MM was dissolved in the solution from Step 2.
4. 320 ml of ethyl alcohol was added to the solution of Step 3.
5. The pH of the solution from Step 4 was adjusted to pH 3.4 ( $\pm$  0.2).
6. Sufficient water (WFI) was added to bring the volume of the solution to 10 liters.
7. Under aseptic conditions, the solution was filtered using two 10" Millipore Durapore 0.2  $\mu$ m filter (CVGL).

8. 5 ml of the filtrate from Step 7 was loaded into 2000 - 20cc multi-dose vials (i.e., 5 ml of filtrate per 20cc vial) and partially stoppered with lyo stoppers.
9. The vials were loaded into a lyophilizer where the temperature was stabilized to 25°C (Dura-Stop MP Freeze Dryer, FTS system).

35 10. Lyophilization Cycle

a. The temperature was measured from the shelf where the loaded vials were placed, and thermocouples were fitted to various vials to measure their temperature.

b. The lyophilization chamber temperature was equilibrated at 25°C. The loaded vials were introduced into the lyophilization chamber and the temperature was maintained at 25°C for 20 minutes.

c. The shelf temperature was decreased from 25°C to -10°C over a period of 2 hours 20 minutes (Ramp rate: -0.25°C/min).

d. The temperature was maintained at -10°C for 2.5 hours.

e. The temperature was decreased to -40°C over 2 hours (Ramp rate:

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-0.25°C/min).

f. The temperature was maintained at -40°C for 5.0 hours.

g. The lyophilization chamber pressure was reduced to 100 mTorr.

h. After the pressure reached 100 mTorr (approximately

5 30 minutes), the temperature was maintained at -40°C for an additional 5 hours.

i. The temperature was increased to -16°C over 8 hours (Ramp rate: 0.05°C/min.) and maintained at -16°C for an additional 10 hours.

j. The temperature was increased to 70°C over 7 hours 10 minutes (Ramp rate: 0.20°C/min) and maintained at 65°C until all the

10 thermocouples indicated a temperature of 60°C for at least 10 hours.

k. The temperature was decreased to 25°C over 3 hours (Ramp rate: -0.25°C/min) and maintained at 25°C for 2 hours.

l. The lyophilization chamber pressure was gradually increased to 4 psi using Nitrogen (NF) over a period of no less than 15 minutes.

m. The vials were stoppered under partial pressure.

n. The lyophilization chamber pressure was increased to atmospheric pressure using filtered air.

11. The vials were then sealed.

20

Characteristic analytical data confirms that the resulting composition contains MM HCl in a crystalline anhydrous form (having X-ray crystallography data as shown in Fig IIB).

**2C. Other Intravenous Formulation Batch Sizes**

25 Similarly, batches for 4000 - 20cc vials, 6000 - 20cc vials or 8000 - 20cc vials can be manufactured for the formulations described in Examples 2A or 2B by increasing the quantity of the ingredients, proportionally.

**2D. Other Intravenous Formulations Varying the Crystalline Anhydrous Salt of MM**

30 By following the procedures described in Examples 2A or 2B and substituting the crystalline anhydrous form of mycophenolate mofetil hydrochloride with the other crystalline anhydrous forms of mycophenolate mofetil (e.g., the sulfate, phosphate and sulfate salts prepared as described in Example 1B) there are obtained the corresponding intravenous formulations.

**EXAMPLE 3**

**ALTERNATE FORMULATION OF  
MYCOPHENOLATE MOFETIL HYDROCHLORIDE**

40 Manufacturing batches of the following formulations containing MM HCl in its crystalline anhydrous forms can be prepared following the procedures described in Example 2.

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Formulation Components	Amount of Ingredient Per Vial	
	Formulation A	Formulation B
Mycophenolate Mofetil (gm)	0.5	0.5
1 N HCl (gm)	1.22	1.22
Polysorbate 80 (mg)	0.07	---
Ethanol, 95% (mL) <sup>a</sup>	0.16	---
WFI <sup>a</sup> , qs to volume	5	4
1 N HCl/1 N NaOH <sup>b</sup>	to pH 3.4	to pH 3.4

10 <sup>a</sup> removed during lyophilization<sup>b</sup> added for pH adjustment

## EXAMPLE 4

DETERMINATION OF FORM  
OF MYCOPHENOLATE MOFETIL HYDROCHLORIDE  
BY X-RAY POWDER DIFFRACTION

15 The crystalline form of MM HCl was determined by X-ray crystallography. The X-ray crystallography data were obtained on a Nicolet X-ray diffractometer equipped with a fine focus tube and a diffracted beam monochromator. X-ray powder diffraction patterns of the compound MM HCl in its crystalline monohydrate and crystalline anhydrous forms were obtained and are presented in Figures IA and IB, respectively.

20 X-ray powder diffraction patterns of compositions containing MM HCl in its amorphous form, MM HCl in its crystalline anhydrous form (with polysorbate 80, Example 3, Formulation A), and MM HCl in its crystalline anhydrous form (without polysorbate 80, Example 3, Formulation B) were obtained and are presented in Figures IIA, IIB and IIC, respectively.

25 The X-ray crystallography data shown in Figure IIB and Figure IIC shows that the formation of the crystalline form of MM HCl in two compositions is not effected by the presence of polysorbate 80, i.e., formation of crystalline anhydrous MM HCl is not impaired by polysorbate 80.

30 The X-ray crystallography data shown in Figures IIB, IIC and IB, shows that the MM HCl in compositions with and without polysorbate 80 are in their crystalline anhydrous form.

## EXAMPLE 5

DETERMINATION OF  
AMORPHOUS AND CRYSTALLINE ANHYDROUS FORMS OF  
MYCOPHENOLATE MOFETIL HYDROCHLORIDE  
BY DIFFERENTIAL SCANNING CALORIMETRY

35 This example describes a method for distinguishing the various crystal forms of MM HCl (e.g., amorphous, monohydrate and anhydrous) using differential scanning calorimetry (DSC). The DSC thermograms were recorded on a Perkins Elmer DSC-7 System. A DSC thermogram represents heat

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flow as a function of temperature, thus providing a measure of a compound's melting point.

The DSC thermogram for the amorphous form of MM HCl (Fig IIIA) shows an exotherm starting at -8.65°C and a melting endotherm starting at 38.50°C that levels at 44.77°C.

On the other hand, the DSC thermogram for the crystalline anhydrous form of MM HCl, after the lyophilization process, (Fig IIIB) shows no phase transitions until melting at 145.41°C to 155.0°C.

The melting point of MM HCl monohydrate is 154-155°C (as disclosed in U.S. Patent No. 4,753,935, issued June 28, 1988 (Example 3).

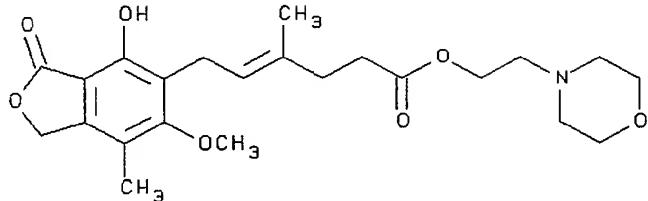
Therefore the amorphous, crystalline monohydrate and crystalline anhydrous forms of MM HCl were readily distinguished by their DSC thermograms.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

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## WHAT IS CLAIMED:

1. A crystalline anhydrous compound of the formula



10 wherein the compound is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate.

2. The compound of Claim 1 wherein the anion is chloride.

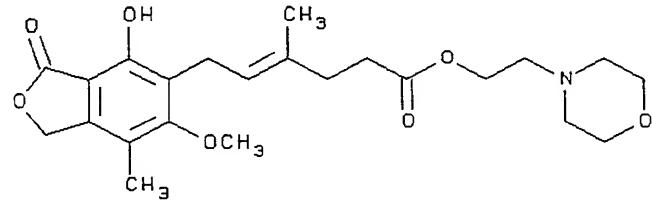
15 3. The crystalline anhydrous compound of Claim 2 characterized by a melting point of about 145 to about 155°C.

20 4. The crystalline anhydrous compound of Claim 3 further characterized by a Differential Scanning Calorimetry thermogram shown in Figure IIIB.

5. The crystalline anhydrous compound of Claim 4 further characterized by the powder X-ray diffraction pattern shown in Figure IB.

25 6. Crystalline anhydrous 2-(4-morpholino)ethyl-E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

30 7. A composition suitable for preparing an aqueous intravenous formulation which comprises a compound of the formula



wherein:

40 the compound is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate, and

said salt is in a crystalline anhydrous form; and pharmaceutically acceptable excipients.

45 8. The composition of Claim 7 wherein the only pharmaceutically

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acceptable excipient is polysorbate 80.

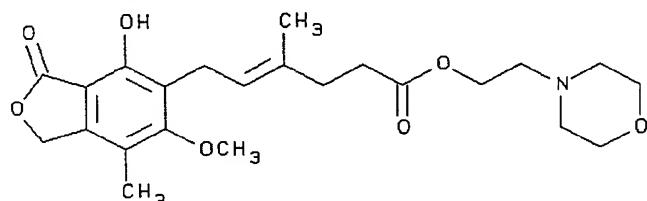
9. The composition of Claim 8 characterized by the powder X-ray diffraction pattern shown in Figure IIB.

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10. The composition of Claim 7 wherein the pharmaceutically acceptable excipients are polysorbate 80 and citric acid.

11. A process for preparing a composition suitable for preparing an intravenous formulation which comprises a compound of the formula

15



wherein:

20 the compound is complexed with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate; and

25 said salt is in a crystalline anhydrous form; and pharmaceutically acceptable excipients;

30 said process comprising:

25 a) compounding said compound and pharmaceutically acceptable excipients to form a bulking solution where the pH of said bulking solution is adjusted to about 3.2 to 3.6,

b) loading said bulking solution into vials, and

c) lyophilizing said loaded vials to form said composition.

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35 12. The process of Claim 11 wherein said lyophilizing step comprises

a) decreasing the temperature over a range of about 25°C to -40°C;

b) decreasing the pressure to about 100 mTorr;

c) increasing the temperature over a range of about -40°C to 70°C; and

d) decreasing the temperature over a range of about 70°C to 25°C.

40 13. The process of Claim 11 wherein said lyophilizing step comprises

a) decreasing the temperature over a range of about 25°C to -10°C at a ramp rate of about -.25°C/min;

b) maintaining temperature at about -10°C for about 2 hours and 30 minutes;

c) decreasing the temperature over a range of about -10°C to -40°C

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at a ramp rate of about -0.25°C/min;

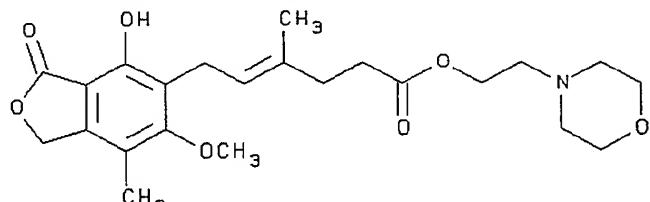
d) decreasing the pressure to about 100mTorr;

e) increasing the temperature over a range of about -40°C to -16°C  
at a ramp rate of about 0.05°C/min;

5 f) increasing the temperature over a range of about -16°C to 70°C  
at a ramp rate of 0.20 °C/min ±0.1°C; and

g) decreasing the temperature over a range of about 70°C to 25°C.

## 14. Use of a compound of the formula

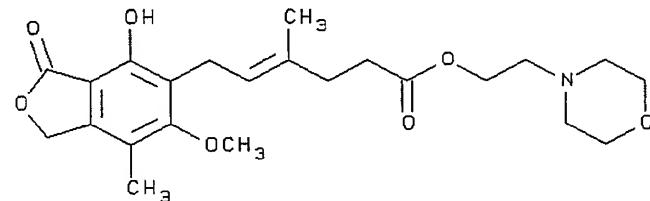


wherein:

the compound is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate, and

said salt is in its crystalline anhydrous form;  
for the treatment of allograft rejection, especially including cardiac allograft rejection, pancreatic allograft rejection and renal allograft rejection, and autoimmune disorders, psoriasis, inflammatory diseases, including in particular rheumatoid arthritis, diabetes, tumors and viral diseases.

## 15. An intravenous formulation prepared by combining a liquid medium and a composition which comprises a compound of the formula



wherein:

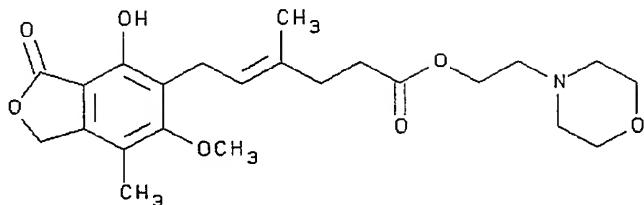
the compound is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate, and

said salt is in its crystalline anhydrous form; and pharmaceutically acceptable excipients.

16. A process for preparing an intravenous formulation comprising  
45 mixing

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5 a) a compound of the formula



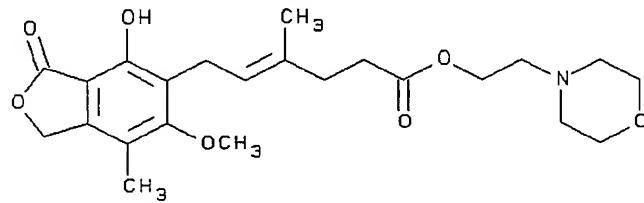
10 wherein:

15 the compound is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate, and

said salt is in its crystalline anhydrous form;

17. A kit useful for preparing an intravenous formulation comprising

20 a) a composition which comprises a compound of the formula



25 wherein:

30 the compound is complexed as a salt with an anion selected from the group consisting of chloride, sulfate, phosphate and acetate, and

said salt is in a crystalline anhydrous form; and pharmaceutically acceptable excipients; and

b) an appropriate amount of a liquid medium.

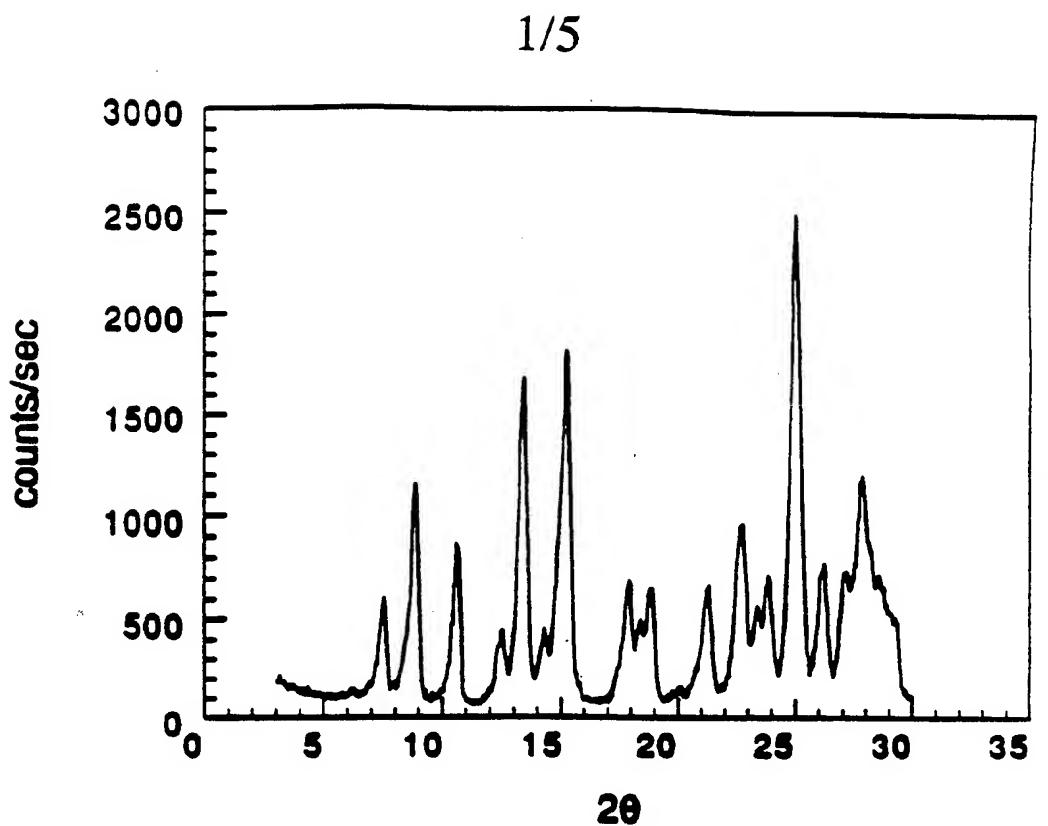


Figure 1A

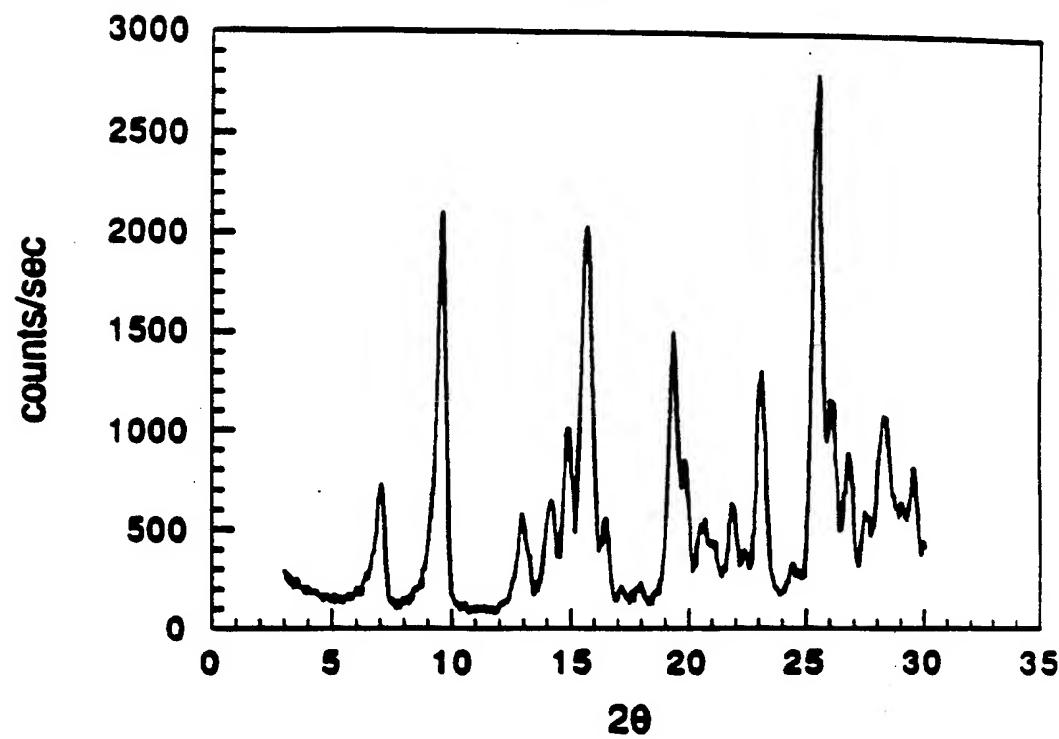


Figure 1B

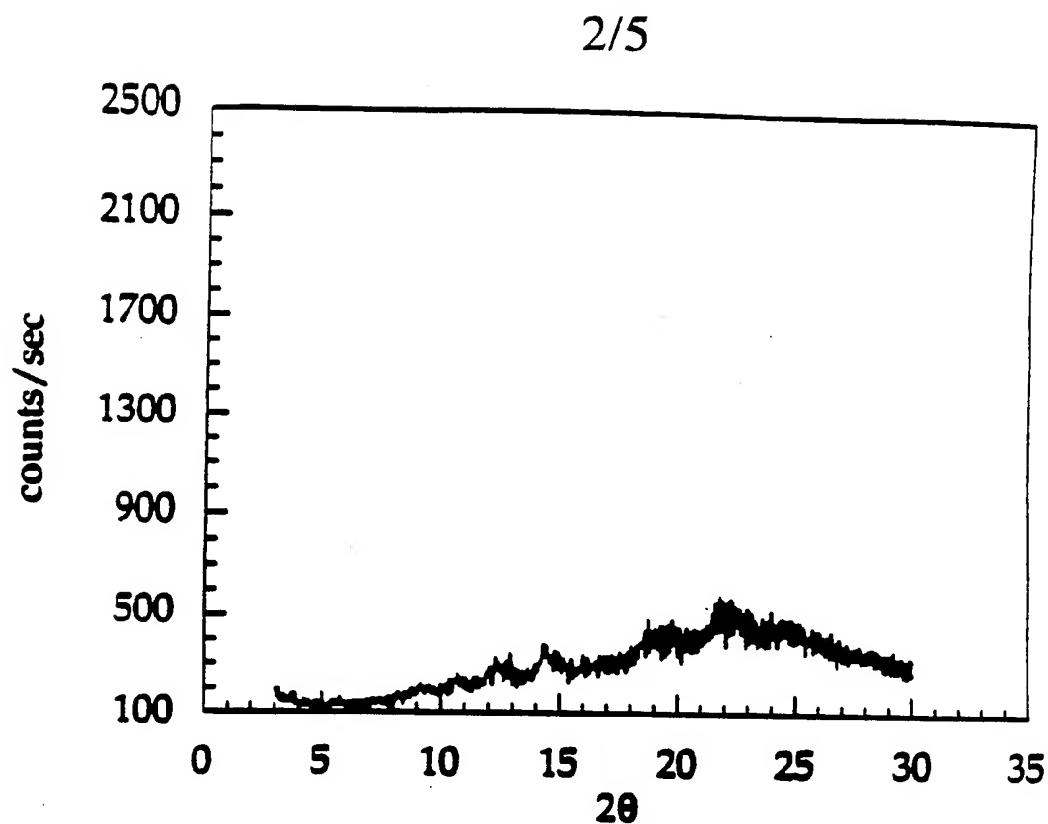


Figure 2A

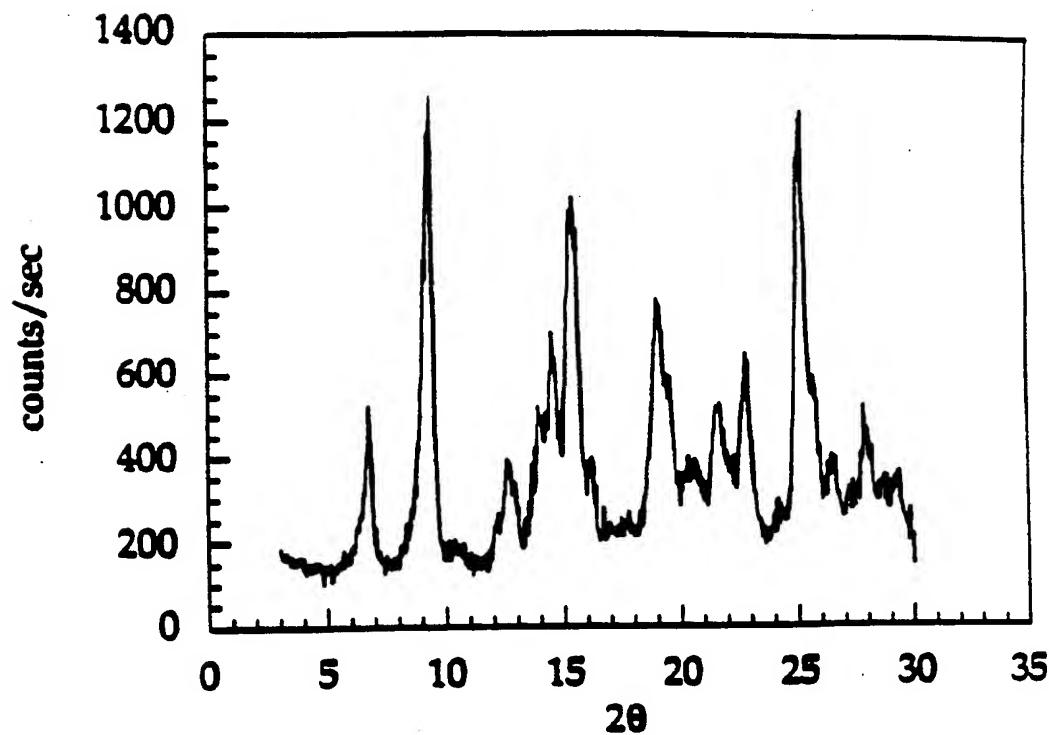


Figure 2B

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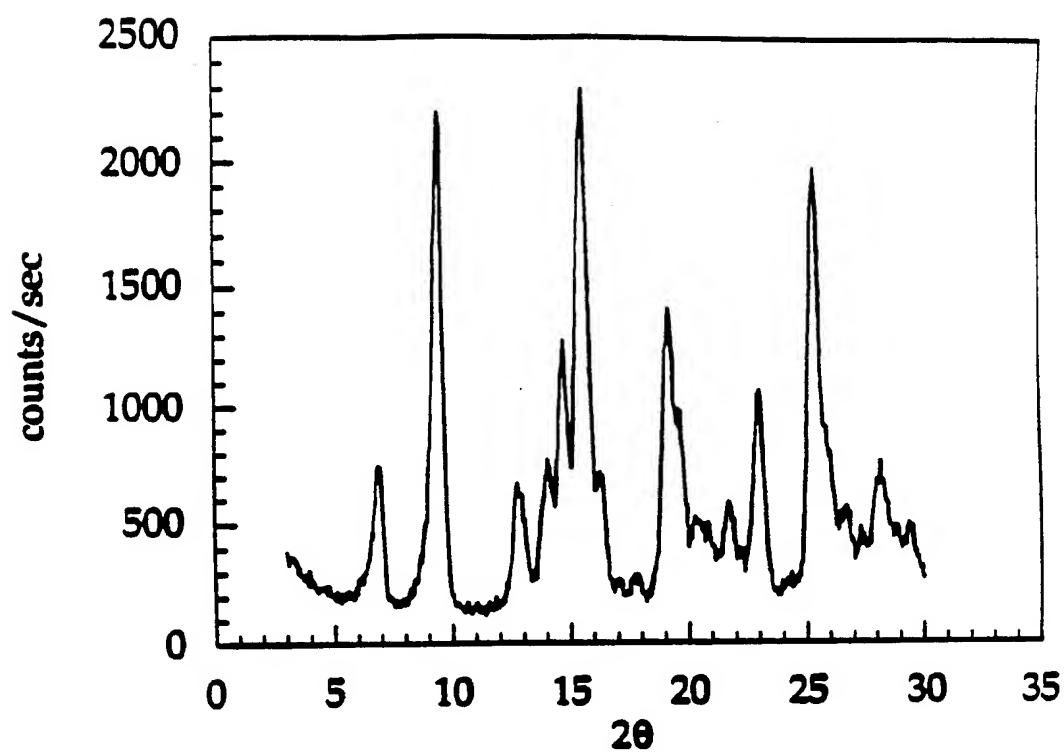


Figure 2C

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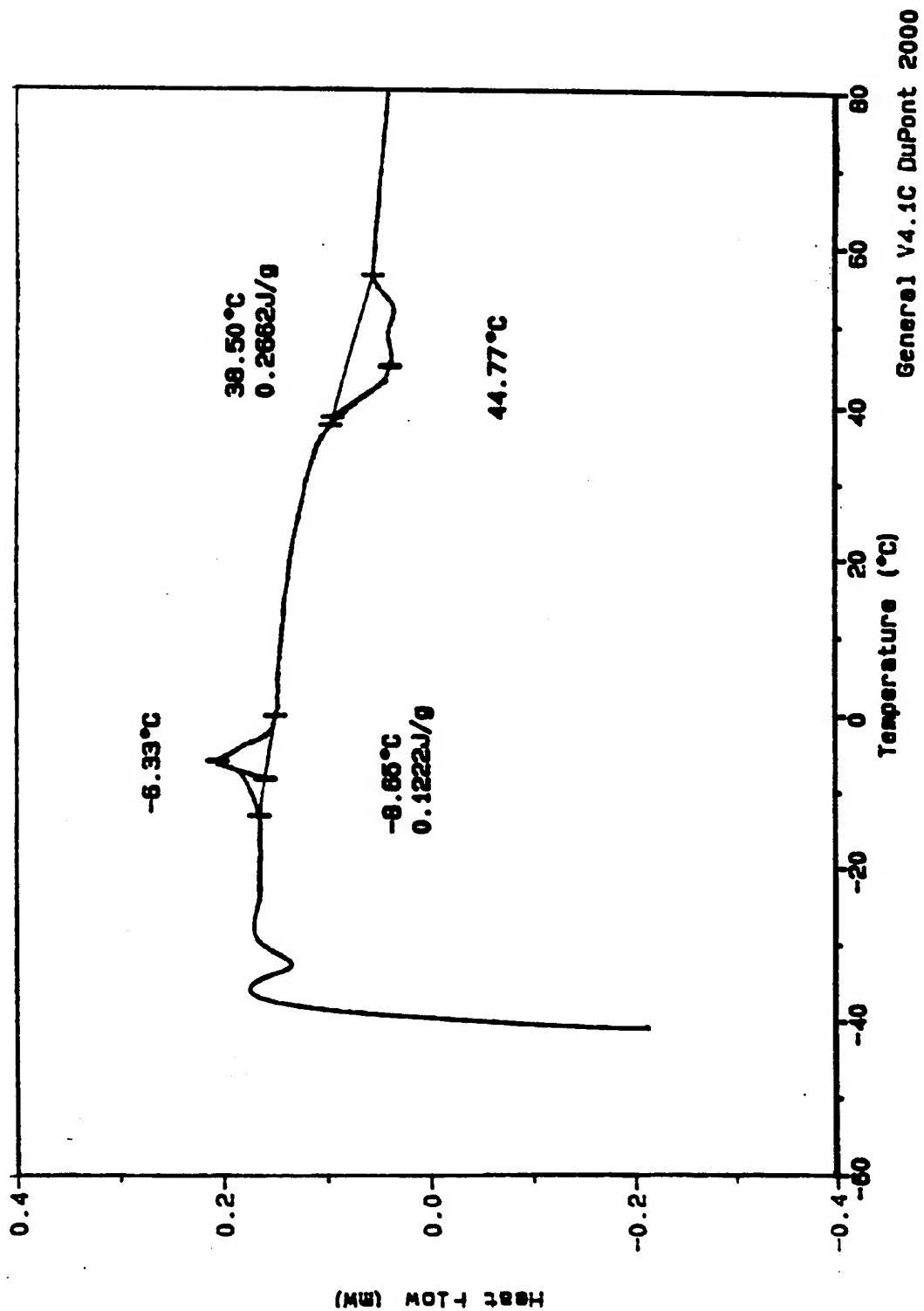


Figure 3A

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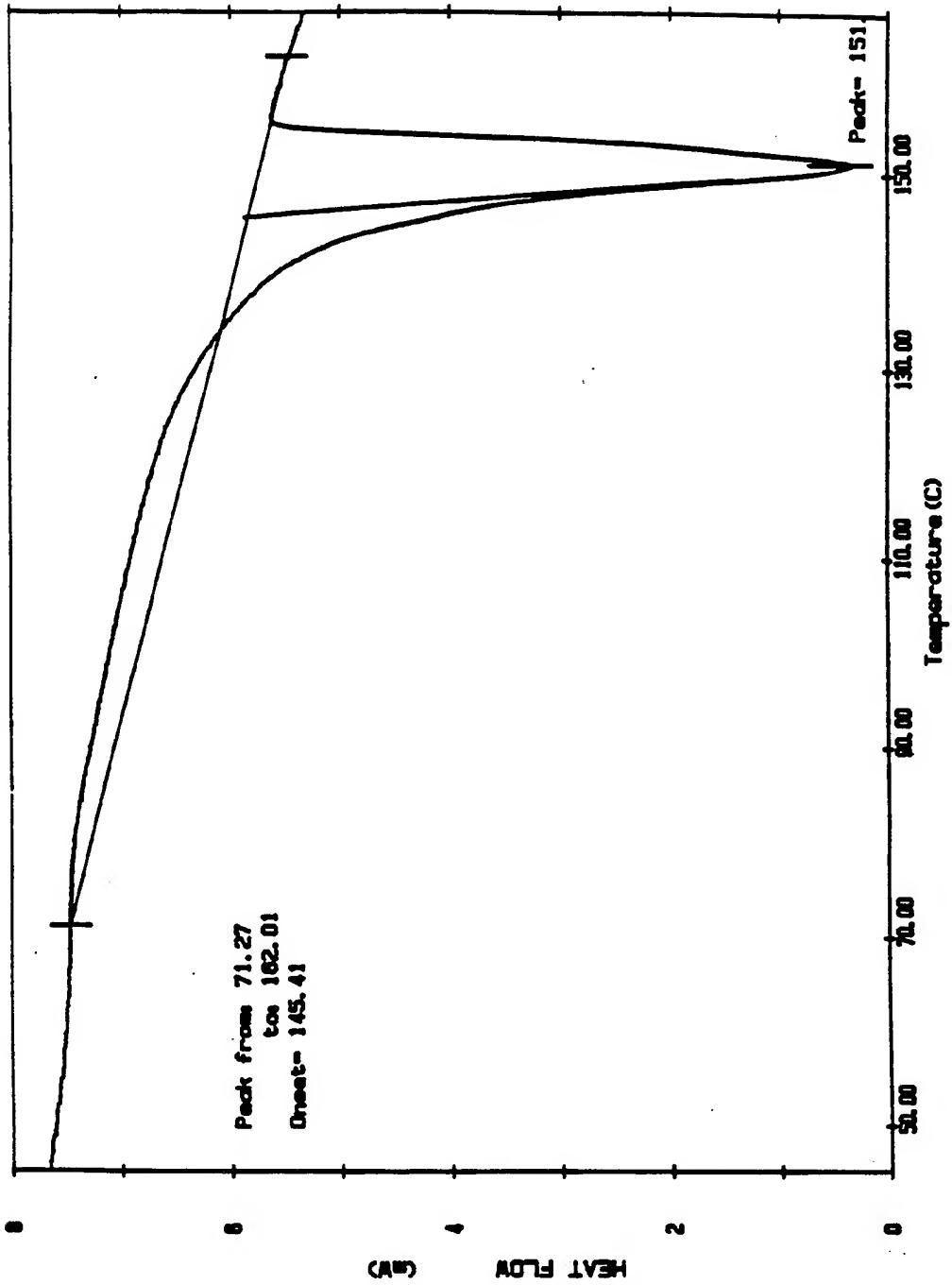


Figure 3B

## INTERNATIONAL SEARCH REPORT

Inte:   Application No  
PCT/US 94/10142A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07D307/88 A61K31/535

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 281 713 (SYNTEX (U.S.A.) INC.) 15 December 1987 see the whole document & US,A,4 753 935 (...) cited in the application -----	1-17

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

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- "E" earlier document but published on or after the international filing date
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1 Date of the actual completion of the international search

13 December 1994

Date of mailing of the international search report

22.12.94

Name and mailing address of the ISA

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Paisdor, B

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 94/10142

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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